

# The TIGR Plant Repeat Databases: a collective resource for the identification of repetitive sequences in plants

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## ABSTRACT

In a number of higher plants, a substantial portion of the genome is composed of repetitive sequences that can hinder genome annotation and sequencing efforts. To better understand the nature of repetitive sequences in plants and provide a resource for identifying such sequences, we constructed databases of repetitive sequences for 12 plant genera: *Arabidopsis*, *Brassica*, *Glycine*, *Hordeum*, *Lotus*, *Lycopersicon*, *Medicago*, *Oryza*, *Solanum*, *Sorghum*, *Triticum* and *Zea* ([www.tigr.org/tdb/e2k1/plant.repeats/index.shtml](http://www.tigr.org/tdb/e2k1/plant.repeats/index.shtml)). The repetitive sequences within each database have been coded into super-classes, classes and sub-classes based on sequence and structure similarity. These databases are available for sequence similarity searches as well as downloadable files either as entire databases or subsets of each database. To further the utility for comparative studies and to provide a resource for searching for repetitive sequences in other genera within these families, repetitive sequences have been combined into four databases to represent the Brassicaceae, Fabaceae, Gramineae and Solanaceae families. Collectively, these databases provide a resource for the identification, classification and analysis of repetitive sequences in plants.

## INTRODUCTION

Although plant genome size varies significantly due to ploidy differences, another contributor to genome size variation is the repetitive DNA content (1). For example, maize (*Zea mays*) has a genome size of 2.3–2.7 Gb (2), yet an estimated 50–80% of the genome is composed of repetitive sequences (1,3). A number of different repetitive sequences have been reported in plants and these can be classified into super-classes, classes and sub-classes based on structure and sequence composition. The transposable element super-class includes retrotransposons, transposons and miniature inverted-repeat transposable elements (MITEs) [for recent review see (4)]. Retrotransposons, which transpose through an RNA

intermediate, include those with long terminal repeats (LTRs) as well as those without LTRs, which are termed long interspersed nuclear elements (LINEs) and short interspersed nuclear elements (SINEs). Plant centromeres are composed of tracts of tandemly repeated sequence, also termed satellite repeats, that are interspersed with other repetitive sequences such as centromeric- and pericentromeric-specific retrotransposons (5,6). Telomeric sequences include the telomere repeat sequence and telomere-associated sequences [for review see (7)]. Another super-class of repetitive sequences are the 18S–5.8S–25S (45S) and 5S ribosomal DNAs (rDNAs), which encode the structural RNA components of ribosomes (8).

The high degree of similarity and duplication of repetitive sequences within certain plant genomes presents difficulties in annotation and genome analyses due to the false associations that can occur. To best identify and catalog the repetitive sequences within plant genomes as part of our overall genome annotation efforts in plants, we created a series of databases from 12 plant genera representing four plant families and made these available for searching and download.

## CONSTRUCTION OF THE REPEAT DATABASES

Repetitive DNA sequences of 12 plant genera (*Arabidopsis*, *Brassica*, *Glycine*, *Hordeum*, *Lotus*, *Lycopersicon*, *Medicago*, *Oryza*, *Solanum*, *Sorghum*, *Triticum* and *Zea*) were retrieved from GenBank and other published records based on their annotation. After elimination of duplicated sequences and trimming of contaminating vector sequences, the sequences were coded into five super-classes: transposable elements, centromere-related, telomere-related, rDNA and unclassified repetitive sequences. These super-classes were then broken down into classes and sub-classes of repeats (Supplementary table 1). The collected repetitive sequences from genera within the Brassicaceae (*Arabidopsis*, *Brassica*), Fabaceae (*Glycine*, *Lotus*, *Medicago*), Gramineae (*Hordeum*, *Oryza*, *Sorghum*, *Triticum*, *Zea*) and Solanaceae (*Lycopersicon*, *Solanum*) families were combined into a repeat database for the plant family. As shown in Table 1, we were able to collect and code 3993 repetitive sequences representing 5.33 Mb of sequence by querying public databases. The majority of these repetitive sequences were obtained from the Gramineae

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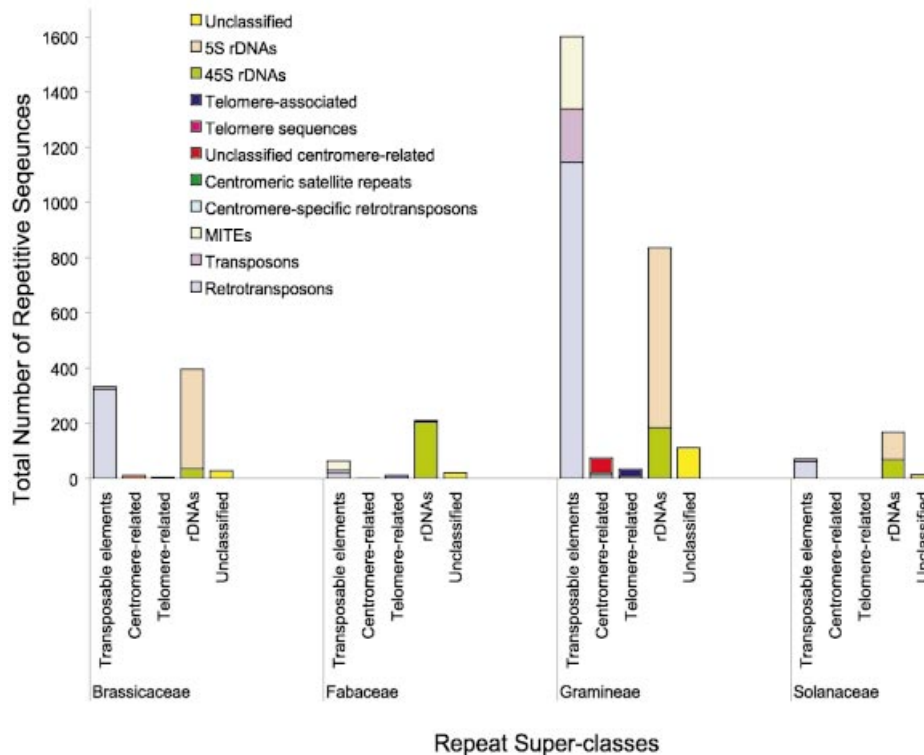
**Table 1.** Statistics of the repetitive sequences within the family repeat databases

Family	No. entries	Length (kb)
Brassicaceae	775	449.5
Fabaceae	308	292.6
Gramineae	2658	4434.8
Solanaceae	252	154.1
Total	3993	5331.0

family [2658 entries (66.6%), representing 4.4 Mb], consistent with previous reports of the high degree of repetitive sequences within Gramineae species such as maize (*Zea mays*) (1,3) and rice (*Oryza sativa*) (9,10). The second highest number of repetitive sequences was obtained from the Brassicaceae family (775 entries, 19.4%), which is reflective of the availability of the entire *Arabidopsis* genome (11). A smaller number of repetitive sequences were obtained from the Fabaceae (308 entries, 7.7%) and Solanaceae (252 entries, 6.3%) families. Throughout the entire set of family databases, the highest number of entries was in the transposable element super-class with a total of 2068 sequences (Fig. 1). Within the transposable element super-class, retrotransposons were the most abundant (1550 entries, ~75% of the total transposable element entries). The second most abundant repeat super-class was the rDNA super-class with 1610 entries in total. Detailed tables showing the composition of each family repeat database can be obtained on the project web page at [www.tigr.org/tdb/e2k1/plant.repeats/rp.stat.shtml](http://www.tigr.org/tdb/e2k1/plant.repeats/rp.stat.shtml).

To capture repetitive sequences in the public databases that were not annotated as repetitive sequences and thus expand the database for a single genus (e.g. *Oryza*), we searched available genomic sequences (the HTGS and PLANT divisions of GenBank) with the nascent family repeat database (e.g. TIGR Gramineae Repeat Database) using FLAST (12). Sequences that matched a repetitive DNA sequence within the family repeat database with ~75% identity and ~95% in overall length were selected and coded accordingly. The sequences were combined with repetitive sequences obtained previously from public databases to create the TIGR Plant Repeat Database for that genus. There are a total of 29 561 entries in the 12 genera-specific repeat databases, representing 15 Mb of sequence, a 3-fold expansion of repetitive sequences compared with our family repeat databases (Table 2; detailed statistics available at [www.tigr.org/tdb/e2k1/plant.repeats/rp.stat.shtml](http://www.tigr.org/tdb/e2k1/plant.repeats/rp.stat.shtml)). The *Oryza* database contains the largest amount of repetitive sequence, measured either by the number of entries or by the length of repetitive sequence, reflecting the abundance of genomic DNA for *Oryza sativa* subsp. *japonica* as part of the International Rice Genome Sequencing Project (13–16).

While the HTGS and PLANT divisions of GenBank are repositories for sequences typically representative of whole genes or large stretches of genomic DNA [e.g. bacterial artificial chromosome (BAC) clones], the genome survey sequences (GSS) division of GenBank is a repository for single-pass sequences such as BAC end sequences and whole-genome shotgun sequences. Although the single pass and short length of GSS sequences have limitations due to sequence

**Figure 1.** Composition of the plant family repeat databases. The numbers for each of the five repeat super-classes and major classes within the four plant family repeat databases are shown.

**Table 2.** Statistics of the repetitive sequences within the 12 genera-specific repeat databases created from searching the HTGS and PLANT divisions of GenBank

Genus	No. entries	Total length (kb)	Total length of sequences searched (Mb) <sup>a</sup>	No. sequences extracted	Total length of sequences extracted (kb)
<i>Arabidopsis</i>	1744	957.2	119.9	1198	588.2
<i>Brassica</i>	249	89.6	2.2	20	9.2
<i>Glycine</i>	130	165.2	3.7	14	9.0
<i>Hordeum</i>	708	670.7	3.0	186	103.0
<i>Lotus</i>	71	42.9	26.4	7	4.9
<i>Lycopersicon</i>	95	71.0	3.2	9	2.3
<i>Medicago</i>	452	222.1	68.7	324	123.7
<i>Oryza</i>	23637	8928.3	502.2	23102	8316.6
<i>Solanum</i>	167	85.9	1.7	1	0.4
<i>Sorghum</i>	233	192.9	1.9	28	6.9
<i>Triticum</i>	783	903.4	2.9	124	58.2
<i>Zea</i>	1292	2746.6	13.5	555	522.3
Total	29561	15075.7	749.3	25568	9744.7

<sup>a</sup>Repetitive sequences were identified by searching the HTGS and PLANT divisions of GenBank with the respective family repeat database, parsing the genomic sequences and collapsing overlapping sequences into one record.

**Table 3.** Summary of repetitive sequences extracted by searching the GSS division of GenBank

Genus	Total length of sequences searched (Mb)	No. of sequences extracted <sup>a</sup>	Total length (kb) of extracted sequences
<i>Arabidopsis</i>	87.9	8018	2809.1
<i>Brassica</i>	386.2	34314	19255.2
<i>Glycine</i>	6.3	149	64.0
<i>Hordeum</i>	0.07	0	0
<i>Lotus</i>	19.8	479	201.1
<i>Lycopersicon</i>	5.8	343	174.9
<i>Medicago</i>	1.9	15	6.6
<i>Oryza</i>	61.5	4627	1989.8
<i>Solanum</i>	0.03	0	0
<i>Sorghum</i>	25.9	1755	464.6
<i>Triticum</i>	0.2	17	7.4
<i>Zea</i>	566.3	124724	68793.0
Total	1161.9	174441	93765.7

<sup>a</sup>Repetitive sequences were identified by searching the GSS division of GenBank with the respective family repeat database, parsing the genomic sequences and collapsing overlapping sequences into one record.

errors and lack of large contiguous stretches of sequence, GSS sequences can provide a broader sampling of an unfinished genome. To further identify repetitive sequences in the 12 plant genera represented in our databases, we searched the GSS division for repetitive sequences using the nascent family repeat sequence databases as the query. As shown in Table 3, 174 441 sequences representing 93.8 Mb were identified in a search of 1162 Mb of GSS sequence. The highest number (124 724) and length of repetitive sequences (69 Mb) identified in the GSS division were from *Zea*, consistent with the high degree of repetitive content in maize (1,3) and the deposition of a large number of sequences from two maize genomic sequencing projects (17). *Brassica* had the second most abundant number and length of repetitive sequences identified from the GSS sequences (34 314 sequences representing 19.2 Mb) attributable to the availability of a large number of whole shotgun sequences for *Brassica oleraceae* (<http://www.tigr.org/tdb/e2k1/bogl1/>). Due to the limited amount of sequence in the GSS division for *Hordeum* and

*Solanum*, no repetitive sequences were identified. The large number and total length of the GSS-derived sequences presents a logistical problem with the incorporation of these repetitive sequences into our database. Thus, we elected to keep the GSS- and HTGS/PLANT-division-derived repetitive sequences separate and not merge these into a single database.

## ACCESS TO THE DATABASES

Several modes of access are available for the TIGR Plant Repeat Databases. All databases, the four family databases, the 12 HTGS/PLANT-division-derived genera-specific databases, and the 10 GSS-division-derived genera-specific databases are available for BLAST searching at <http://tigrblast.tigr.org/euk-blast/index.cgi?project=plant.repeats>. All of these databases (26 in total) can be downloaded through anonymous FTP as a flat file ([ftp://ftp.tigr.org/pub/data/TIGR\\_Plant\\_Repeats/](ftp://ftp.tigr.org/pub/data/TIGR_Plant_Repeats/)). For repetitive sequences obtained by querying GenBank using annotation, the header line contains the internal

repeat code classification, the originating GenBank accession number and description. For sequences obtained through similarity searches, the header contains the internal repeat code classification, the GenBank GI number, the coordinate information of the parent sequence and the classification of the extracted repeat sequence. Additionally, the user can query the four family databases and 12 HTGS/PLANT-division-derived genera-specific databases for a subset of sequences (<http://www.tigr.org/tdb/e2k1/plant.repeats/subset.shtml>). Available selection criteria include the database, super-class or class of repetitive sequences, as well as sequences derived from querying GenBank by annotation or sequences derived by sequence similarity searches.

## CONCLUSIONS

We have generated a set of repetitive sequence databases that represent four major plant families and a number of agriculturally significant plant genera including maize (*Zea*), rice (*Oryza*), wheat (*Triticum*), barley (*Hordeum*), sorghum (*Sorghum*), soybean (*Glycine*), tomato (*Lycopersicon*), potato (*Solanum*) and canola (*Brassica*). Model species such as *Arabidopsis thaliana*, *Lotus japonicus* and *Medicago truncatula* are represented in the *Arabidopsis*, *Lotus* and *Medicago* databases, respectively. The collective set of databases contains >100 Mb of repetitive sequence and with the ongoing genome sequencing efforts in plants, these databases can be expanded in the future. The broad nature of the databases with respect to taxon provides a starting point for the identification of repetitive sequences in additional species within these families.

## SUPPLEMENTARY MATERIAL

Supplementary Material is available at NAR Online.

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## REFERENCES

1. Flavell, R.B., Bennett, M.D., Smith, J.B. and Smith, D.B. (1974) Genome size and the proportion of repeated nucleotide sequence DNA in plants. *Biochem. Genet.*, **12**, 257–269.
2. Arumuganathan, K. and Earle, E.D. (1991) Nuclear DNA content of some important plant species. *Plant Mol. Biol. Rep.*, **9**, 208–218.
3. SanMiguel, P., Tikhonov, A., Jin, Y.K., Motchoulskaia, N., Zakharov, D., Melake-Berhan, A., Springer, P.S., Edwards, K.J., Lee, M., Avramova, Z. *et al.* (1996) Nested retrotransposons in the intergenic regions of the maize genome. *Science*, **274**, 765–768.
4. Feschotte, C., Jiang, N. and Wessler, S.R. (2002) Plant transposable elements: where genetics meets genomics. *Nature Rev. Genet.*, **3**, 329–341.
5. Nagaki, K., Song, J., Stupar, R.M., Parokony, A.S., Yuan, Q., Ouyang, S., Liu, J., Hsiao, J., Jones, K.M., Dawe, R.K. *et al.* (2003) Molecular and cytological analyses of large tracks of centromeric DNA reveal the structure and evolutionary dynamics of maize centromeres. *Genetics*, **163**, 759–770.
6. Copenhagen, G.P., Nickel, K., Kuromori, T., Benito, M.I., Kaul, S., Lin, X., Bevan, M., Murphy, G., Harris, B., Parnell, L.D. *et al.* (1999) Genetic definition and sequence analysis of *Arabidopsis* centromeres. *Science*, **286**, 2468–2474.
7. McKnight, T.D., Fitzgerald, M.S. and Shippen, D.E. (1997) Plant telomeres and telomerases. A review. *Biochemistry*, **62**, 1432–1441.
8. Shishido, R., Sano, Y. and Fukui, K. (2000) Ribosomal DNAs: an exception to the conservation of gene order in rice genomes. *Mol. Gen. Genet.*, **263**, 586–591.
9. Deshpande, V.G., Ranjekar, P.K. (1988) Repetitive DNA in three Gramineae species with low DNA content. *Hoppe Seylers Z. Physiol. Chem.*, **361**, 1223–1233.
10. McCouch, S.R., Kochert, G., Yu, Z.H., Wang, Z.Y., Khush, G.S., Coffman, W.R. and Tanksley, S.D. (1988) Molecular mapping of rice chromosomes. *Theor. Appl. Genet.*, **76**, 815–829.
11. The *Arabidopsis* Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature*, **408**, 796–815.
12. Yuan, Q., Quackenbush, J., Sultana, R., Pertea, M., Salzberg, S.L. and Buell, C.R. (2001) Rice bioinformatics. Analysis of rice sequence data and leveraging the data to other plant species. *Plant Physiol.*, **125**, 1166–1174.
13. Feng, Q., Zhang, Y., Hao, P., Wang, S., Fu, G., Huang, Y., Li, Y., Zhu, J., Liu, Y., Hu, X. *et al.* (2002) Sequence and analysis of rice chromosome 4. *Nature*, **420**, 316–320.
14. The Rice Chromosome 10 Sequencing Consortium (2003) In-depth view of structure, activity and evolution of rice chromosome 10. *Science*, **300**, 1566–1569.
15. Sasaki, T., Matsumoto, T., Yamamoto, K., Sakata, K., Baba, T., Katayose, Y., Wu, J., Niimura, Y., Cheng, Z., Nagamura, Y. *et al.* (2002) The genome sequence and structure of rice chromosome 1. *Nature*, **420**, 312–316.
16. Sasaki, T. and Burr, B. (2000) International Rice Genome Sequencing Project: the effort to completely sequence the rice genome. *Curr. Opin. Plant Biol.*, **3**, 138–141.
17. Chandler, V.L. and Brendel, V. (2002) The maize genome sequencing project. *Plant Physiol.*, **130**, 1594–1597.